AWARD NUMBER: W81XWH-13-1-0380

TITLE: The Role of the New mTOR Complex, mTORC2, in Autism Spectrum Disorders

PRINCIPAL INVESTIGATOR: Mauro Costa-Mattioli

CONTRACTING ORGANIZATION: Baylor College of Medicine
Houston, TX 77030

REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
October 2016

2. REPORT TYPE
Annual

3. DATES COVERED
30Sep2015 - 29Sep2016

4. TITLE AND SUBTITLE

The Role of the New mTOR Complex, mTORC2, in Autism Spectrum Disorders

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0380

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Mauro Costa-Mattioli

E-Mail:costamat@bcm.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor College of Medicine
One Baylor Plaza
Houston, TX 77030-3498

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of my DOD-supported research is to determine the role of the new mTOR complexes (mTORC1 and mTORC2) in Autism Spectrum Disorder (ASD). Individuals with ASD exhibit impaired social interactions, repetitive abnormal repetitive behavior as well as cognitive problems. In addition, a large proportion of ASD individual suffer from seizures. Autism is a heritable genetically heterogeneous disorder and mutations in the negative regulator of the mammalian target of rapamycin (mTOR) signaling pathway PTEN were associated with ASD. However, little is known about the mechanism underlying Pten-induced pathology. Here, we show that in the hippocampus of *pten* fb-KO mice - where Pten is conditionally deleted in the murine forebrain - the activity of both mTORC1 and mTORC2 is increased. In addition, we found that *pten* fb-KO mice exhibit seizures, learning and memory and ASD-like. Interestingly, genetic dissection of mTOR complexes function *in vivo* reveals that mTORC1 is responsible for the enlarged brain phenotype whereas mTORC2 regulates EEG seizures, learning and memory as well ASD-like phenotypes in *pten*-deficient mice. Moreover, we found that mTORC2 regulates these processes by controlling glucose metabolism. Our new insights hold promise for new specific mTORC2-based treatments for ASD and related mTORopathies.

15. SUBJECT TERMS

Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	13	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

1. Introduction: Autism Spectrum Disorder (ASD) is one of the most common neurological disorders worldwide, characterized by abnormal social interaction, deficits in communication and restricted/repetitive stereotype behaviors and, In addition, a significant number of autistic individuals suffer from intellectual disability and seizures[1-4]. Single gene mutations are linked to ASD and dysregulation of Mechanistic Target Of Rapamycin (mTOR) signaling cascade has been linked to ASD[5-7]. More specifically, loss-of function mutations of the phosphatase and tensin homolog (PTEN), a negative regulator of mTOR signaling, were associated with syndromic ASD[8]. While in PTEN-deficient individuals (or mouse models), the activity of both mTOR complexes (mTORC1 and mTORC2) is up-regulated, it is generally believed that persistent increased mTORC1 activity leads ASD-like symptoms[9]. However, most of the evidence supporting a role for mTORC1 in ASD relies heavily on the chronic pharmacological inhibition of mTOR by rapamcyin, which blocks the activity of both mTORC1 and mTORC2 complexes in brain. There are no drugs to specifically target mTORC1 or mTORC2. Thus, the scope of this research was to determine the role of mTOR complexes in a pten-deficient mouse model of ASD using mouse genetics. To investigate the selective involvement of mTORC1 and/or mTORC2 in pten-associated ASD, we used the Cre-loxP system and generated mice lacking a) pten (pten fb-KO mice), b) pten and raptor (a defining component of mTORC1; pten-raptor fb-KO mice) and c) pten and rictor (a defining component of mTORC2) in the murine forebrain.

2. Keywords: Autism Spectrum Disorder (ASD), mTORC2, mTORC1, protein synthesis, actin polymerization, metabolism, long-term memory, social behaviors, repetitive behaviors, seizures.

3. Overall Project Summary.

The major goal of our grant application was to elucidate the molecular and cellular mechanisms underlying ASD, with a special emphasis on the mTOR signaling pathway and its two major complexes. We believe that in the three years of funding, we have made remarkable progress. Specifically, we have discovered that each mTOR complex differentially contributes to different aspects of ASD. While the activity of both mTOR complexes (mTORC1 and mTORC2) is increased in pten-deficient individuals and mice[8], the individual contribution of mTOR complexes to the molecular, behavioral and electrophysiological abnormalities associated with pten deficiency remains unknown. We found that mice lacking pten in forebrain neurons (pten fb-KO) exhibit increased mTORC1 and mTORC2. In addition, pten fb-KO mice show enhanced brain size, ASD-like behaviors (including social and cognitive deficits as well as repetitive behaviors), seizures and early mortality. We found that genetic deletion of mTORC1 (raptor), but not mTORC2 (rictor), only restores normal brain size. Surprisingly, genetic silencing of mTORC2, but not mTORC1, in pten-deficient mice, prolonged lifespan, suppressed seizures, rescued the cognitive and ASD-like behaviors as well as the seizure phenotype. These insights hold promise for new specific mTORC2-based treatments for ASD and related mTORopathies.

Selective genetic silencing of mTORC2 or mTORC1 in pten-deficient mice. As previously reported, we found that mTORC2-mediated phosphorylation of Akt at Ser473 (an established readout of mTORC2 activity[6]) and mTORC1-mediated phosphorylation of ribosome protein S6 (an established readout of mTORC1 activity)[10] were both increased in the hippocampus of *pten* fb-KO mice (**Fig. 1e-g**). We found that while in the hippocampus of *pten-rictor* fb-DKO mice, mTORC1 activity remained abnormally up-regulated, mTORC2 activity was restored. By contrast, in *pten-raptor* fb-DKO mice, the opposite is true, namely mTORC2 activity remains up-regulated by mTORC1 activity is normalized (**Fig. 1**). *Hence, conditional deletion of rictor selectively blocks mTORC2 activity in pten-deficient neurons and conditional deletion of raptor selectively block mTORC1 activity in pten-deficient neurons*. These loss-of-function manipulations allow us to dissect the functional role of each mTOR complex in pten-deficient-mediated pathology.

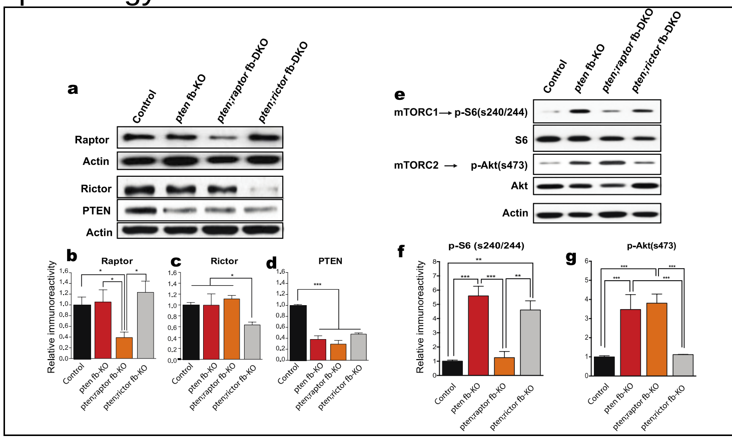

Fig. 1. mTORC2 activity is selectively suppressed in the hippocampus from *pten-rictor* fb-KO mice, whereas mTORC1 activity is selectively suppressed in the hippocampus of pten-raptor fb-DKO. Western blots showing the levels of raptor (**a,b**), rictor (**a,c**), pten (**a,d**), mTORC1 activity (**e,f;** p-S6 Ser240/244) and mTORC2 activity (**e,g;** p-Akt at Ser473) in the hippocampus of control, pten fb-KO mice, pten, raptor fb-KO mice and, pten, rictor fb-KO mice (n=5 per group)

Genetic silencing of mTORC1, but not mTORC2, restore brain size in pten-deficient mice. A significant percentage of children with ASD exhibit brain enlargement[1], which becomes noticeable a few months after birth and persists until early adolescence. However, little is not about the molecular mechanism underlying the early brain overgrowth[11,12]. Moreover, it is currently unknown whether the same mechanism regulating brain size also controls the other core features of ASD, including the seizures and behavioral and electrophysiological abnormalities.

To measure the contribution of each mTOR complex to the brain size in pten-deficient mice, we measured the size of the brain at 4 weeks postnatal. Deletion of *pten* in the postnatal forebrain leads to an increased brain size, compared to control littermates (**Fig. 2a-b**). Interestingly, genetic silencing of mTORC1, but not mTORC2, restores brain size in *pten*-deficient mice (**Fig. 2a-b**). *Thus, the exacerbated increased in mTORC1 (but not mTORC2) activity accounts for the increased brain size in pten-deficient mice.*

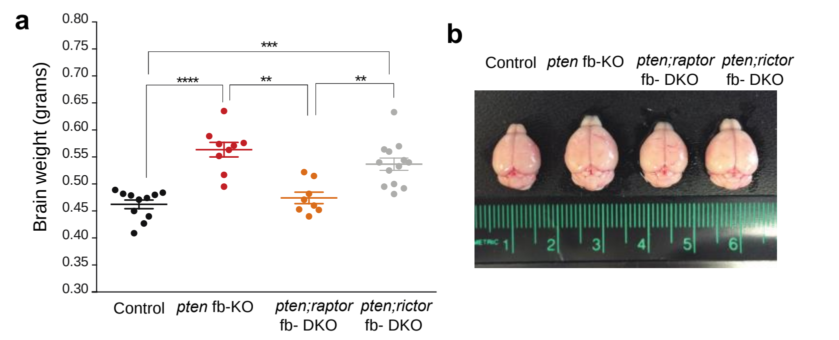

Fig. 2. mTORC1, but not mTORC2, regulates macrocephaly in pten-deficient mice. a. Brain mass is significantly increased in pten fb-KO mice but resoted to normal when mTORC1, but not mTORC2, activity is silenced (n=8-11). **b.** Representative views of brains from male control, pten fb-KO, *pten;raptor* fb-KO and *pten;rictor* fb-KO mice.

<u>Genetic silencing of mTORC2, but not mTORC1, prolonged survival in pten-deficient mice</u>. Not unexpectedly, Kaplan-Meier analysis of animal survival revealed a dramatic decreased in survival in *pten* fb-KO mice compared to control mice (**Fig. 3).** The majority of the *pten* fb-KO mice die a few weeks postnatal. Interestingly, genetic suppression of mTORC1 had no a major effect on *pten* fb-KO mice survival (compare *pten* fb-KO mice vs. *pten;raptor* DKO survival curves). Remarkably, genetic suppression of mTORC2 significantly extended survival (almost three times) in pten-deficient mice (compare *pten* fb-KO mice vs. *pten;rictor* DKO survival curves**; Fig. 3;** *pten;rictor* fb-DKO die at an age of 119.4 +/- 25). *Hence, selective inhibition of mTORC2, but not mTORC1, in Pten-deficient mice prolongs their survival.*

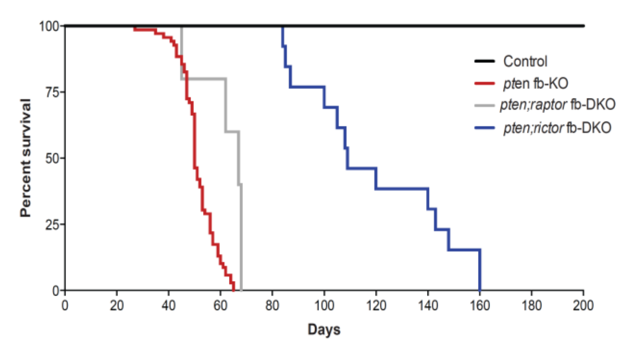

Fig. 3. Genetic inhibition of mTORC2 prolongs survival in *Pten*-deficient mice. Kaplan-Meier survival curves for wild-type, Pten fb-KO, Pten-raptor fb-DKO and Pten-rictor fb-DKO. The median survival for *Pten-rictor* fb-DKO (n=35) is 120 days, a 140% extension in life span compared with the single Pten- fb-DKO mice (n=11) or Pten-raptor fb-DKO (n=8). The curves of *Pten* fb-KO mice and *Pten-rictor* DKO mice are significantly different (p<0.0001).

<u>Genetic silencing of mTORC2, but not mTORC1, suppressed seizures and runaway hyperexcitabilty in *pten*-deficient mice.</u>

Because both human individuals and mouse models with Pten mutations exhibit epilepsy[8], we next analyzed spontaneous seizures and abnormal electroencephalogram (EEG) activity. We found that *pten* fb-KO mice show abnormal EEG and behaviorl seizures (**Fig. 4**). Consistent with their survival rates, *pten;raptor* fb-DKO mice also showed tonic-clonic and EEG seizures. By contrast, *pten-rictor* fb-DKO mice showed only some abnormal interictal spikes in the EEG pattern but not EEG or behavioral seizures (**Fig. 4**). *Thus, inhibition of mTORC2, but not mTORC1, suppresses the seizures EEG phenotype in Pten-deficient mice.*

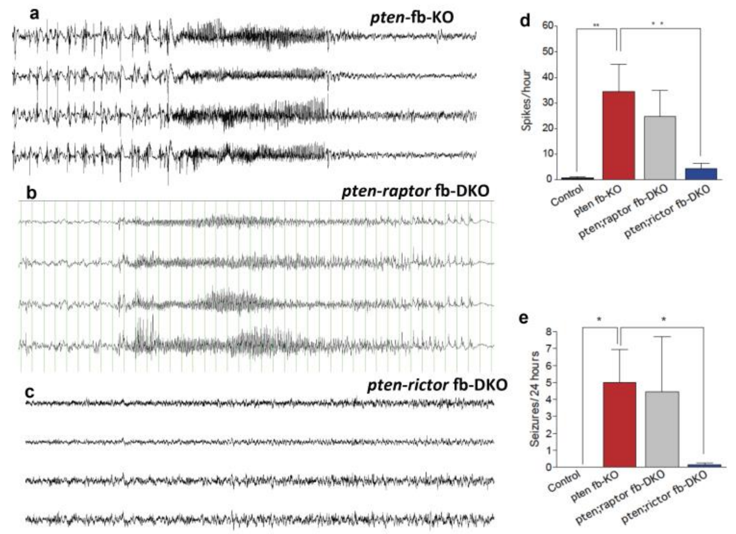

Fig. 4. Genetic inhibition of mTORC2, but not mTORC1, suppresses EEG seizures *Pten* fb-KO mice. Traces from bilateral cortical electrodes (Left hemisphere, L, Right hemisphere, R) show abnormal EEG seizures in freely moving *pten* fb-KO mice (**a;** n=6) and *pten-raptor* fb-DKO mice (**b,** n=3). In *Pten-rictor* fb-DKO mice EEG seizures are suppressed which are suppressed in (**c;** n=5). d,e, Quantification of spikes and seizure frequencies.

Given the increased excitability in the brain network of pten-deficient mice, we next examined whether intrinsic excitability is altered in hippocampal pyramidal neurons. To this end, we performed whole-cell recordings in slices, as we previously described[13]. Neurons fired significantly more action potentials to equivalent step injections. Interestingly, such an increased in runaway excitability is restored in pten-deficient neurons lacking rictor, but not raptor (**Fig. 5**). These data indicate that the increased runaway excitability is due to increased mTORC2, but not mTORC1, activity.

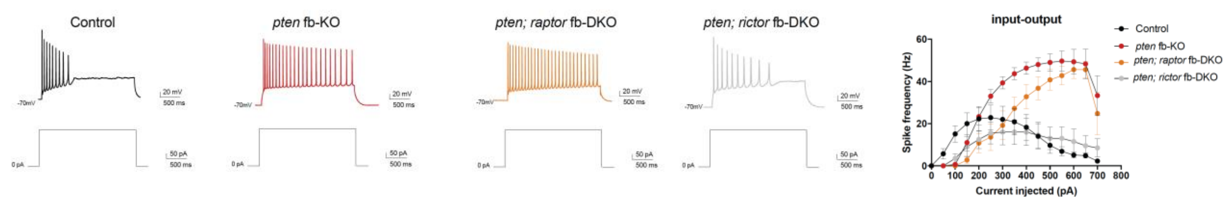

Fig. 5. Genetic inhibition of mTORC2, but not mTORC1, suppresses the increased intrinsic runaway excitability. Examples of voltage traces elicited by current injection in hippocampal pyramidal neurons from control, pten fb-KO mice, pten;raptor fb DKO mice and pten;rictor fb-DKO mice. b) Quantification of spike frequency vs current injection (n=6=10 per group).

Because in *pten;raptor* fb-DKO mice genetic inhibition of mTORC1 had not effect on seizures onset and duration, hyperexcitability and animal survival, we focused on the behavioral characterization of *pten-rictor* deficient mice.

Genetic silencing of mTORC2 rescues long-term memory deficits in pten-deficient mice. Emotions have a powerful impact on memory; most vivid autographical memories tend to be of emotional effects. In addition, 70-80% of autistic individuals suffer from mental retardation[1-3]. Thus, we first tested emotional memory, as we previously described[13,14]. To this end, mice were studied in contextual Pavlovian fear conditioning. Contextual fear conditioning, and hippocampus-dependent task, was induced by pairing a context (conditioned stimulus; CS) with a foot shock (the unconditioned stimulus; US). Mice were subsequently exposed to the auditory tone or visual stimulus (CS) and fear responses [mouse stop moving ("freezes")] were taken as an index of the strength of

memory (**Fig. 6a**). As expected, compared to control mice, *pten* fb-KO mice showed a dramatic reduction in freezing behavior 24 hr post-training, indicating that their long-term fear memory is impaired (**Fig. 6b).** Strikingly, long-term memory is significantly improved once *rictor* is deleted in *pten*-deficient mice. *Thus, silencing mTORC2 activity restores LTM in mice lacking pten.*

We next studied object recognition memory, another hippocampal dependent task. In this task, an object is presented to the subject mouse. After a 24 hr delay, the object is presented again with a new object (**Fig. 6c).** The time spent exploring each object is tracked via by a computer-operated optical animal activity system (ANIMAZE). We found that Pten-deficient mice failed to discriminate between and old and a new object (**Fig. 6d**). However, genetic deletion of rictor restore the object recognition long-term memory deficits in Pten-deficient mice. *Taken together, these data indicate that inhibiton of mTORC2, but not mTORC1, restore hippocampal-dependent long-term memroy formation in pten-deficient mice.*

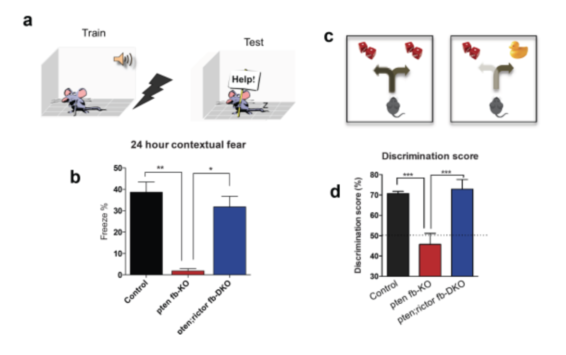

Fig. 6. Genetic inhibition of mTORC2 rescues the deficient long-term fear and object recognition memory in Pten fb-KO mice. a, Schematic of experimental design. **b,** For contextual fear conditioning, freezing times were recorded 24 hr after conditioning. As compared to control mice, Pten fb-KO mice show deficient freezing 24 hr after training, indicating that their long-term memory is impaired. In Pten-rictor fb-DKO mice, freezing levels are similar to those observed in WT mice, indicating that their long-term memory is restored. **c,** Schematic of object recognition task. **d)** Discrimination scores between two objects.

<u>Genetic silencing of mTORC2 rescues social behaviors in pten-deficient mice.</u> Given that social interaction deficits are salient features of ASD individuals[2], we next studied social behaviors. First, we assessed reciprocal social interactions by recording the amount of time a pair of mice spent interacting in a neutral arena[15] (**Fig. 7a**), as we previously described[5]. We found that compared to control mice, *pten*-deficient mice showed reduced reciprocal interaction (**Fig. 7b**). Interestingly, in *pten-rictor* fb-KO mice, reciprocal social interaction is normal. Next, we measured sociability and preference for social novelty using the Crawley 3-chamber test[15] (**Fig. 7c, 7e**). In the sociability task, we compared the time a mouse spends interacting with an empty wired cage and one containing a mouse (**Fig. 5c**); whereas in the social novelty test, we measured the time a mouse spends interacting with a familiar or a stranger mouse (**Fig. 7e**). Consistent with the direct social interaction results, we found that pten fb-KO mice had normal sociability (**Fig. 7d**), but showed no preference for interaction with a stranger versus a familiar mouse in the social novelty test (**Fig. 7f**). Strikingly, deletion of mTORC2 rescues the social novelty deficits in *pten*-deficient mice (**Fig. 7f**). *Taken together these data indicate that pten-fb KO mice display social deficits and inhibition of mTORC2 restored their phenotypes.*

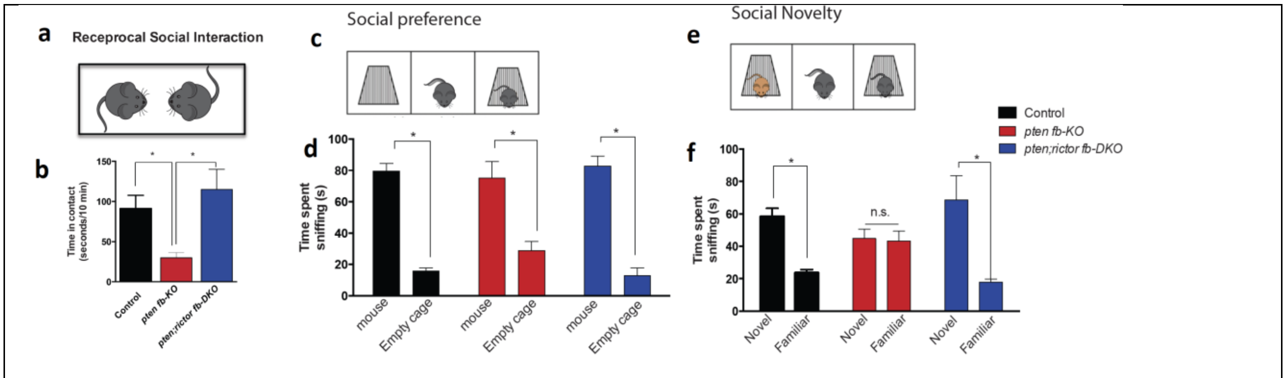

Fig. 7. Social behaviors are normal in Pten-rictor fb-DKO mice. a) Schemetic of reciprocal social interaction task**. b,** Compared to control or pten-rictor fb-DKO mice, pten-fb KO mice showed reduced reciprocal interaction. **c, e,** Schematic of three chamber social interaction task. **f,** Control mice spent most of the time interacting with the stranger mouse but *Pten* fb-KO mice spent equal time interacting with the familiar or strange mouse, indicating that social behavior is impaired in these mice. Like WT mice, *Pten-rictor* fb-KO mice spent more time interacting with the stranger mouse, indicating that social behavior is restored.

Genetic silencing of mTORC2 rescues repetitive behaviors in *pten*-deficient mice.
Because ASD patients also exhibit repetitive/stereotyped behaviors, we first assessed marble burying, as we previously described[5]. In this task, mice were individually placed in Plexiglas cages containing 5 cm deep fresh bedding, with 20 black marbles pre-arranged in 5x4 evenly spaced rows (**Fig. 8a**). Testing was conducted for 20 min. After the test period, unburied marbles were counted. We found that *pten*-deficient mice buried more marbles than control mice, indicating repetitive behavior (**Fig. 8b**). Remarkably, deletion of *rictor* (mTORC2) restored the repetitive behavior in pten-deficient mice (**Fig. 8b**). We next assessed behavioral flexibility in the T-maze. In this task, normal animals tend to investigate first one and the other arm of the maze (**Fig. 8c**). Notably, *pten*-deficient mice explored the same arm of the maze, demonstrating and impairment in behavioral flexibility (**Fig. 8d**). Remarkably, in *pten-rictor* fb-DKO mice the behavioral inflexibility behavior is restored (**Fig. 8d**). In conclusion, *our data demonstrate that inhibition of mTORC2 restores memory, social and repetitive behaviors in pten-deficient mice*.

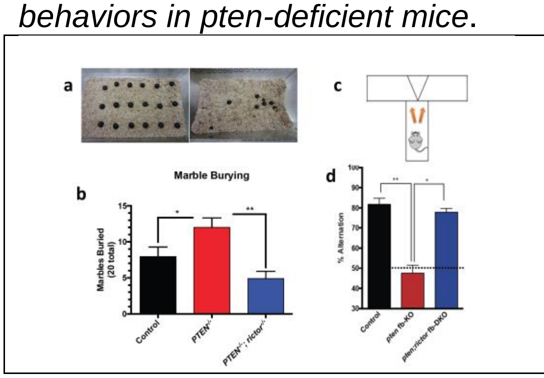

Fig. 8. Behavioral flexibility and repetitive behaviors are restored by deletion of mTORC2 in Pten-deficient mice. a) Scheme of the marble burying task. **b)** Quantification of stereotype marble burying behavior **c)** Schematic of the T maze. **d)** Alternation scores (%) across 10 sessions of testing in the spontaneous alternation task. (n=7-9 per group). Data are mean ± s.e.m. *$P < 0.05$, **$P < 0.01$.

Genetic inhibition of mTORC2, but not mTORC1, restores key metabolic changes in *pten*-deficient mice. PTEN regulates changes in metabolism by blocking the Warburg effect [16,17], a process in which “cancer” cells catabolize large amounts of

glucose through glycolysis. To explore whether the same is true in neurons lacking *pten*, we perform metabolomics from hippocampal samples from control and pten deficient mice. Consistent with the idea that PTEN regulates metabolic changes associated with and anti-Warburg effect, we found that *pten*-deficient neurons there is a significant increased in metabolites involved in the glycolytic pathway. Remarkably, suppression of mTORC2, but not mTORC1, activity was sufficient to restore these changes in metabolism associated with the loss of *pten* (**Fig. 9**). *Hence, increased mTORC2, but not mTORC1, activity leads to changes in neuronal metabolism associated with the Warburg effect.*

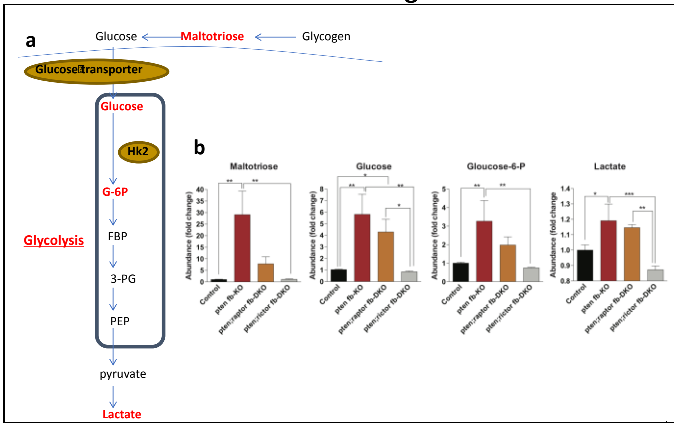

Fig. 9. Metabolic changes are restored by deletion of mTORC2, but not mTORC1, in Pten-deficient mice. **a**) Schematic of the glycolytic pathway. **b)** Metablomic analysis revealed that metabolites in the glycolytic pathway are increased in pten-deficient mice, but restore to normal when mTORC2, but not mTORC1, is deleted (n=6 per group).

4. Key Research Accomplishment

- We developed a novel way to selectively silence mTORC2 activity in *pten*-deficient mice.
- We developed a novel way to specifically silence mTORC1 activity in *pten*-deficient mice.
- We found that genetic deletion of mTORC1 selectively restores brain size in *pten*-deficient mice.
- We found that genetic deletion of mTORC2 prolongs the survival of *pten*-deficient mice.
- We found that genetic silencing of mTORC2 dramatically suppressed seizures in *pten* -deficient mice.
- We found that genetic deletion of mTORC2 improves cognitive and social phenotypes in *pten*-deficient mice.
- We found that genetic deletion of mTORC2 improves repetitive behaviors in *pten*-deficient mice.
- We found that genetic silencing of mTORC1 failed to restore animal's survival and seizures phenotype.
- We found that genetic silencing of mTORC2, but not mTORC1, restores the brain metabolic changes in pten-deficient mice.

5. Conclusion

It has been proposed that increased mTORC1 is responsible for the cellular, synaptic and behavioral abnormalities associated with ASD[8,18-23]. In addition, it is generally believed that a common molecular mechanism regulates both the anatomical (brain size) and core features (behavioral symptoms) of ASD [24]. Our new data challenge these

views by providing causal evidence that mTOR complexes differentially regulate these processes. We found that a) the up-regulation of mTORC1 is only responsible for the enlarged brain size, whereas, b) persistent activation of mTORC2 activity leads to cognitive decline, ASD-like behaviors, seizures and changes in brain metabolism in the Pten-ASD mouse model. Hence, we identified a new signaling pathway (mTORC2) crucially involved in ASD and seizure disorders. Our results may lead to the development of new treatments for ASD and seizure disorders.

Future experiments: Given that a) metabolites in the glycolytic pathway are increased in the brain of *pten* fb-KO mice and b) inhibition of mTORC2 IN pten-deficient mice reverses not only the metabolic changes, but also the synaptic, network and behavioral abnormalities, in future experiments, we will attempt to reduce glycolysis by subjecting *pten*-deficient mice to a ketogenic diet [25]. This diet will reduce the availability of glucose, which is the principal metabolite for glycolysis. Thus, we expect that ketogenic diet will ameliorate some of the symptoms in pten-deficient mice.

6. Publications

a. List all manuscripts submitted for publication during the period covered by this report resulting from this project.

Some of these results described above were presented in

- “Catastrophic Epilepsy” at the Neurological Research Institute (NRI), Houston, Texas
- Symposium Society for Neuroscience (see 1: Huber KM, Klann E, Costa-Mattioli M, Zukin RS. Dysregulation of Mammalian Target of Rapamycin Signaling in Mouse Models of Autism. J Neurosci. 2015 Oct 14;35(41):13836-42. doi: 10.1523/JNEUROSCI.2656-15.2015. PubMed PMID: 26468183; PubMed Central PMCID: PMC4604222.)
- PI3K-mTOR-PTEN Network in Health and Disease, Cold Spring Harbor, 2016.
- Marine Biological Laboratory Course Wood hole, 2016.
- Federation of Latin American and Caribbean Neuroscience, Buenos Aires, Argentina, 2016.
- UT Health Neuroscience symposium, 2016.
- Rush and Helen Record Neuroscience Retreat, 2017.

7. Inventions, Patents and Licenses.

We plan to file and application claiming that blockage of mTORC2 reduce seizures and ameliorate ASD-like symptoms.

8. Reportable Outcome

Nothing to report

9. Other achievements

We developed forebrain-specific Pten double KO mice.
We developed forebrain-specific rictor-Pten double KO mice.
We developed forebrain-specific raptor-Pten double KO mice.

10. References

1 Fombonne, E. The epidemiology of autism: a review. *Psychological medicine* **29**, 769-786 (1999).

2 Mefford, H. C., Batshaw, M. L. & Hoffman, E. P. Genomics, intellectual disability, and autism. *N Engl J Med* **366**, 733-743, doi:10.1056/NEJMra1114194 (2012).

3 Toro, R. *et al.* Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. *Trends Genet* **26**, 363-372, doi:S0168-9525(10)00107-1 [pii]10.1016/j.tig.2010.05.007 (2010).

4 Ghacibeh, G. A. & Fields, C. Interictal epileptiform activity and autism. *Epilepsy & behavior : E&B* **47**, 158-162, doi:10.1016/j.yebeh.2015.02.025 (2015).

5 Buffington, S. A. *et al.* Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell* **165**, 1762-1775, doi:10.1016/j.cell.2016.06.001 (2016).

6 Costa-Mattioli, M. & Monteggia, L. M. mTOR complexes in neurodevelopmental and neuropsychiatric disorders. *Nature neuroscience* **16**, 1537-1543, doi:10.1038/nn.3546 (2013).

7 Hoeffer, C. A. & Klann, E. mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends in neurosciences* **33**, 67-75, doi:S0166-2236(09)00187-8 [pii]10.1016/j.tins.2009.11.003 (2010).

8 Zhou, J. & Parada, L. F. PTEN signaling in autism spectrum disorders. *Current opinion in neurobiology*, doi:S0959-4388(12)00083-9 [pii]10.1016/j.conb.2012.05.004 (2012).

9 Kelleher, R. J., 3rd & Bear, M. F. The autistic neuron: troubled translation? *Cell* **135**, 401-406, doi:S0092-8674(08)01308-1 [pii]10.1016/j.cell.2008.10.017 (2008).

10 Ma, X. M. & Blenis, J. Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* **10**, 307-318, doi:nrm2672 [pii]10.1038/nrm2672 (2009).

11 Hu, W. F., Chahrour, M. H. & Walsh, C. A. The diverse genetic landscape of neurodevelopmental disorders. *Annual review of genomics and human genetics* **15**, 195-213, doi:10.1146/annurev-genom-090413-025600 (2014).

12 Mirzaa, G. M. & Poduri, A. Megalencephaly and hemimegalencephaly: breakthroughs in molecular etiology. *American journal of medical genetics. Part C, Seminars in medical genetics* **166C**, 156-172, doi:10.1002/ajmg.c.31401 (2014).

13 Zhu, P. J. *et al.* Suppression of PKR promotes network excitability and enhanced cognition by interferon-gamma-mediated disinhibition. *Cell* **147**, 1384-1396, doi:S0092-8674(11)01375-4 [pii]10.1016/j.cell.2011.11.029 (2011).

14 Huang, W. *et al.* mTORC2 controls actin polymerization required for consolidation of long-term memory. *Nature neuroscience* **16**, 441-448, doi:10.1038/nn.3351 (2013).

15 Silverman, J. L., Yang, M., Lord, C. & Crawley, J. N. Behavioural phenotyping assays for mouse models of autism. *Nature reviews. Neuroscience* **11**, 490-502, doi:nrn2851 [pii]10.1038/nrn2851 (2010).

16 Garcia-Cao, I. *et al.* Systemic elevation of PTEN induces a tumor-suppressive metabolic state. *Cell* **149**, 49-62, doi:10.1016/j.cell.2012.02.030 (2012).

17 Ortega-Molina, A. & Serrano, M. PTEN in cancer, metabolism, and aging. *Trends in endocrinology and metabolism: TEM* **24**, 184-189, doi:10.1016/j.tem.2012.11.002 (2013).

18 Ehninger, D. *et al.* Reversal of learning deficits in a Tsc2(+/-) mouse model of tuberous sclerosis. *Nature medicine* (2008).

19 Ehninger, D., de Vries, P. J. & Silva, A. J. From mTOR to cognition: molecular and cellular mechanisms of cognitive impairments in tuberous sclerosis. *Journal of intellectual disability research : JIDR* **53**, 838-851, doi:JIR1208 [pii]10.1111/j.1365-2788.2009.01208.x (2009).

20 Ehninger, D. & Silva, A. J. Rapamycin for treating Tuberous sclerosis and Autism spectrum disorders. *Trends in molecular medicine* **17**, 78-87, doi:S1471-4914(10)00148-6 [pii]10.1016/j.molmed.2010.10.002 (2011).
21 Carson, R. P., Van Nielen, D. L., Winzenburger, P. A. & Ess, K. C. Neuronal and glia abnormalities in Tsc1-deficient forebrain and partial rescue by rapamycin. *Neurobiology of disease* **45**, 369-380, doi:S0969-9961(11)00290-7 [pii]10.1016/j.nbd.2011.08.024 (2012).
22 Tsai, P. T. *et al.* Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature* **488**, 647-651, doi:nature11310 [pii]10.1038/nature11310 (2012).
23 Wong, M. Mammalian target of rapamycin (mTOR) inhibition as a potential antiepileptogenic therapy: From tuberous sclerosis to common acquired epilepsies. *Epilepsia* **51**, 27-36, doi:EPI2341 [pii]10.1111/j.1528-1167.2009.02341.x (2011).
24 Ehninger, D., Li, W., Fox, K., Stryker, M. P. & Silva, A. J. Reversing neurodevelopmental disorders in adults. *Neuron* **60**, 950-960, doi:S0896-6273(08)01055-6 [pii]10.1016/j.neuron.2008.12.007 (2008).
25 Schwartz, L., Seyfried, T., Alfarouk, K. O., Da Veiga Moreira, J. & Fais, S. Out of Warburg effect: An effective cancer treatment targeting the tumor specific metabolism and dysregulated pH. *Seminars in cancer biology*, doi:10.1016/j.semcancer.2017.01.005 (2017).

11. Appendices

N/A